Development and Evaluation of Novel Implantable Nanosensors for Real-Time Monitoring of Individual Cells and Cellular Signaling

AFOSR Project Number FA9550-04-1-0248

Final Performance Report

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for the

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I. Objectives

The goal of this project was to develop and demonstrate the potential of a versatile class of nano-biosensors capable of being implanted within individual living cells for the direct conversion of biochemical signaling events into electronic information. In order to monitor the various biochemical species responsible for intracellular signaling (e.g., proteins), with minimal stress to the cell during analysis, we are developing nanobead sensors between 200 and 500 nm in diameter that are capable of being non-invasively positioned at different locations within a living cell via optical tweezers. These sensors employ surface enhanced Raman spectroscopy (SERS) and allow for the qualitative as well as quantitative monitoring of the expression of the specific proteins required for cellular response. Monitoring intracellular signaling species in this fashion allows for the detection of the earliest possible signals associated with a stimulation event, as well as allow for the differentiation of various events from one another at the earliest possible time.

In addition to demonstrating the potential of these sensors for the simultaneous monitoring of multiple signaling species, a secondary goal of this project is to demonstrate the ability to position these sensors to various locations of interest within a cell using optical tweezers. As the long-term goal of these sensors is to be able to study how cells respond to various stimuli, it is important to be able to position them at locations of interest within a cell (e.g., nucleus, cytoplasm, etc.). Therefore, a near infrared (NIR) optical tweezers system is being constructed, in this work. Particular emphasis is being placed on minimizing the laser power necessary for positioning of the SERS-based protein nanosensors, as they will include a partially reflective metal coating surrounding a silica nanosphere core, and will require a unique optical geometry to allow effective positioning.

While these sensors will be widely applicable to the understanding of many different biological reaction pathways, the particular application being addressed in this work is the detection and understanding of early intracellular changes associated with an immunological response following contact with biological agents (i.e., toxins and pathogenic organisms). Since intracellular signals within T lymphocytes are among the earliest potential biological responses to toxins and other infectious agents, detection of an immunological response at this stage as well as

differentiation of one toxin from another, based on cellular response, could dramatically improve survival rates for those exposed. It is for this reason that T-cell activation is the model system for our demonstration of these sensors' potential.

II. Status of Effort

Successful accomplishment of all goals for this project has been accomplished in the nine months of this seedling effort. In particular, the potential of these sensors for the simultaneous detection of cellular level concentrations of as many as 100 different intracellular signaling species (i.e., proteins) has been demonstrated along with their ability to be positioned non-invasively via optical tweezers as well as the ability to distinguish between different doses of biological agent/toxin exposure based upon cellular response. In this research, we have advanced many different areas of scientific research associated with real-time intracellular signaling, including: (1) SERS-based nanosensor development and characterization for label-free protein monitoring, (2) development and optimization of a non-invasive optical tweezers/ multispectral imaging system for positoning and monitoring partially reflective 200 nanometer sized objects, (3) evaluation of T-cell response to external stimuli via conventional bioassays, and (4) practical implantation techniques for insertion of nanosensors into viable cells. Specifically, in the area of nanosensor development and characterization, we have: 1) developed a variation on a common method for producing monodisperse nanospheres, that allows for stable and highly-reproducible controlled diameter silica nanospheres to be fabricated, 2) fabricated and evaluated functional surface enhanced Raman scattering (SERS)-based protein immuno-nanosensors for both IL-2 and insulin both intra- and extra-cellularly and 3) optimized the signal –to-noise of the nanosensors by developing a novel multi-layered sensor geometry capable of enhancing SERS signals by over an order of magnitude. In terms of optical trapping, a NIR optical positioning system has been developed by the co-PI Alexander at ARL, capable of trapping individual nanosphere sensors in cellular environments, as well as obtaining SERS spectra from the sensors. Using this system, cellular background signals have been characterized and cellular analyses have been performed with these nanosensors, demonstrating their potential for real-time intracellular signal monitoring of as many as 100 different protein species simultaneously. In terms of biological response of T-cells to external stimuli, extensive studies

have been performed monitoring IL-2 production following exposure to various external stimulants by the co-PI Ostrand-Rosenberg and co-workers. This work has demonstrated a dose dependent response from the T-cells of IL-2, which can be correlated to the amount of *Staphylococcus aureus* enterotoxin B (SEB) to which the cell has been exposed. Studies employing IL-2 based and phosphorylated ZAP-70 based implantable nanosensors have confirmed the ability of these sensors to track cellular signaling pathways. Finally, the non-toxic and stressing nature of these implantable nanosensors was demonstrated by monitoring the cell viability and routine cellular function of dishes of cultured cells for several days following nanosensor insertion.

III. Accomplishments/New Findings

The research performed during the nine months of this seedling effort have resulted in advances in many different areas of cellular signal monitoring (i.e., nanosensor fabrication and evaluation, SERS substrates for enhanced signal generation, optical tweezers system development, and cellular response function studies), resulting in the successful accomplishment of all of the major goals outlined in the proposal as well as demonstration of the potential of these sensors for the monitoring of real-time signaling events.

During this seedling effort, several scientifically novel accomplishments/discoveries were made in the area of SERS-based nanosensor development for the label-free detection of intracellular proteins. One of the first major accomplishments for this project, was the development of a variation on chemical synthesis protocols for the fabrication of monodisperse nanospheres, that allowed for the production of monodisperse nanospheres with significantly greater control over sphere diameter and with greater precision over previous methods. This allowed for the development of sensors with a more accurate quantitative nature. By modifying a standard sol-gel condensation procedure reported by Stöber et. al. to include a pH controlled reaction inhibitor, we are now capable of preparing silica nanospheres with diameters ranging from 100 nm to 5000 nm with extremely accurate control of size, and a standard deviation of diameter of less than 5%. Characterization of these nanoparticles has been performed using both conventional white light microscopy for the larger diameter spheres (> 500 nm) and scanning

electron microscopy (SEM) for the smaller particles (100 nm – 500 nm). Figure 1 shows a microscopic image of a batch of micrometer diameter spheres produced via this modified method. From this figure the well defined shape of the sphere, due to surface adhesion during synthesis, can be easily seen as well as the monodisperse nature of the process even for the larger size silica nanospheres. Modifying these reaction conditions not only provided a means of accurately controlling the diameter and monodisperse nature of the silica nanospheres produced, but it also allowed for concentration of the nanospheres via a simple centrifugation and decanting steps, making it easier to implement on a larger scale. Additionally, we have investigated the possibility of providing an enhanced surface roughness to these spheres, for additional SERS enhancement, by surface etching with hydrofluoric acid (HF). In this process, HF is added to the condensation reaction in small, picomolar to micromolar, concentrations. Adding the HF prior to the addition of the tetraethyl orthosilicate (TEOS) and allowing it to disperse homogenously throughout the reaction mixture resulted in an equal degree of pitting on the different nanosensors, providing an increased roughness, with minimal sensor-to-sensor variation, as determined via SERS calibrations with standard SERS active chemical compounds.

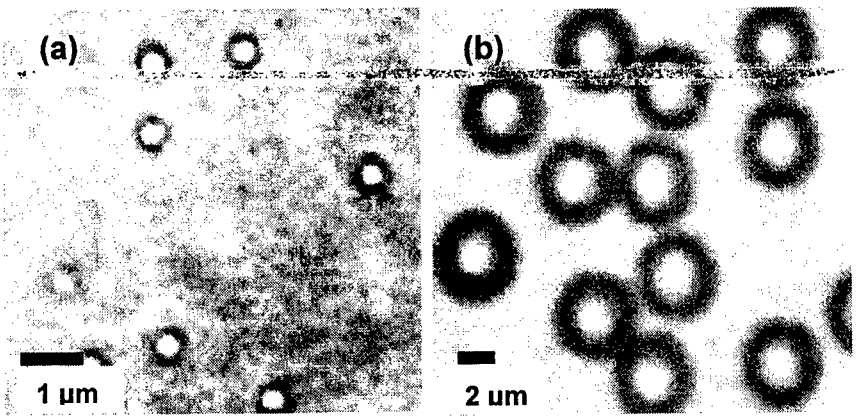

Figure 1: Monodisperse silica microspheres of different diameters. a) ~450 nm and b) ~4500 nm.

In addition to advances in the fabrication of the nanosphere support matrices, this seedling effort has resulted in the development, optimization and evaluation of the first SERS-based immuno-nanosensors for intracellular analyses. These sensors, employ the silica nanoparticles described previously, to provide a core particle for positioning and identification under microscopic for quantitative analyses. They are coated with a layer of silver, monoclonal antibodies for the protein species of interest are attached to the silver surface via short, rigid linking molecules. During this research effort, a great deal of time and effort has been spent in determining the optimal means of depositing silver for the SERS enhancement on the surface of

these nanosphere sensors, as well as the optimal silver thickness, and geometry. This investigation was performed by despositing silver of different linear deposition thickness ranging from 10 nm – 200 nm on 200 nm and 450 nm diameter silica spheres either via a chemical deposition process (i.e., Tollens reaction) in which the entire surface of the sphere is covered, or a vapor deposition process in which only one half of the sphere's surface is covered. From these studies, it was found that both methods provided a simple and effective means of coating thin layers of silver on the nanospheres, however, the chemical deposition method provided several background spectral bands associated with impurities from the deposition process. These background bands dramatically reduced the number of different potential sensors that could be monitored simultaneously within a cellular environment and therefore, vapor deposition was used for the fabrication of all future SERS-based nanosensors.

When depositing the silver via vacuum evaporation, a novel multi-layer deposition process was discovered is capable of enhancing SERS signals by over an order of magnitude relative to comparable single layer SERS substrates with the same total amount of silver deposited. This work resulted in a secondary study into this novel multi-layer effect that has resulted in several publications in scientific journals as well as several research talks at national and international conferences. From these studies, it was determined that the optimal number of metal layers on the silica surface ranged from 2-3, with a total silver thickness of 200 nm. These conditions provided the greatest SERS signal intensity while still allowing light to pass through the silver layer, which is important for positioning of the sensors using the NIR optical tweezers system developed in this work. This multilayer sensor geometry provided enough enhancement to allow the half coated vapor deposited sensors to have a sensitive enough detection capability to monitor only a few copies of a protein, which is crucial for intracellular analyses in a single cell.

Following optimization of the silver layer thickness, the optimal binding process for attachment of the monoclonal antibodies was investigated. In these studies, several different length linker molecules were investigated, each being bound to the silver surface through a thiol linkage. These linker molecules included 2-mercapto-4-methyl-5-thiazole acetic acid (MMTA), 2-amino-6-thiocyanate-benzothiazole (ATB) and 16-mercapto-hexadecanoic acid (MHA) (see

Figure 2). These three crosslinkers represent one short slightly flexible crosslinker (MMT), one short inflexible crosslinker (ATB) and one long flexible crosslinker (MHA). While the shortest crosslinkers were expected to provide the antigen with the closest proximity to the roughened metal surface, and resultingly the greatest SERS enhancement, the long flexible crosslinker was investigated to determine if the steric hindrance of the short multiple crosslinkers and antibodies would keep the antigen from approaching the metal surface. From these experiments it was found that both MMT and ATB allowed for sensors to be developed with equivalent SERS signals for the antigen being evaluated, both being significantly better than the sensors fabricated using the MHA crosslinker. These studies also allowed us to verify that upon binding of the antigen to the crosslinkers, the activity of the antibodies was retained. This antibody activity was studied via direct SERS monitoring of the antibody upon binding as well as independent fluorescence based analyses, in which fluorescently labeled antibodies were bound to the surface of the spheres, and the fluorescence intensity of the remaining unbound antibodies washed off in a constant volume wash were measured. From these fluorescent analyses (Figure 3), the solutions with the least fluorescence represent the best binding of the antibodies to the nanosensors.

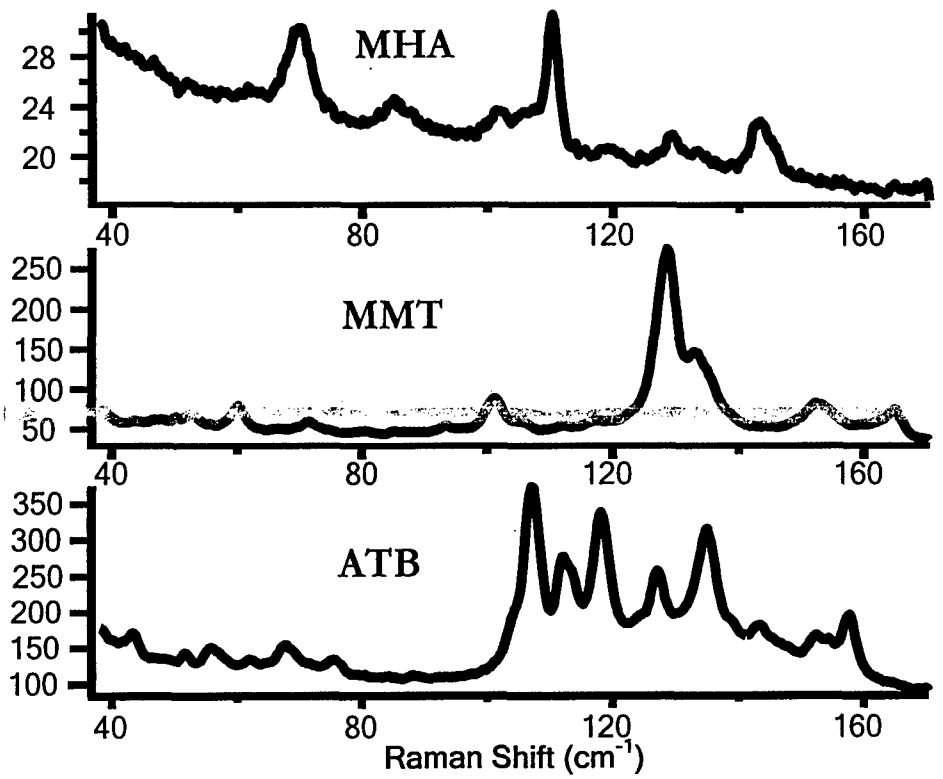

Figure 2: SERS spectra of cross linking molecules MHA, MMT and ATB, bound to individual SERS nanosensors

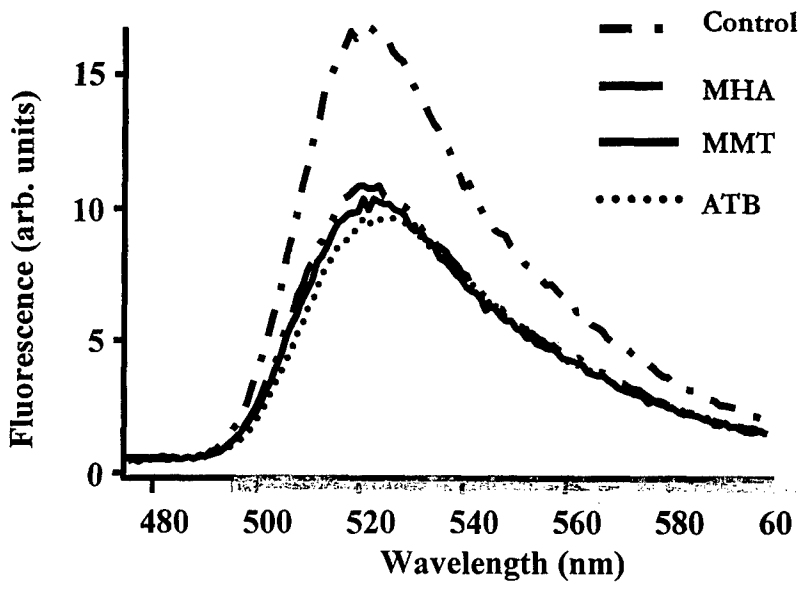

Figure 3: Independent fluorescence evaluation of antibody binding to silver nanospheres. All crosslinkers exhibit significant binding compared to a control in which non-specific binding was evaluated.

In addition to simply monitoring the binding efficiency of the various crosslinkers, the activity of the antibodies were also determined, in order to ensure that the generic binding process used for antibody attachment allowed was widely applicable from antibody to antibody without affecting the specificity and binding affinity of them. From these results, it was found that in all the antibodies studied (i.e., anti-IL-2, anti-human insulin, anti-phosphorylated ZAP-70, and anti-fluorescein) all three of the different crosslinking agents could be used and still retain antibody activity and specificity. This is due to the attachment of the crosslinkers at free amine or carboxyl groups in the heavy invariable portion of the antibodies, thereby not affecting the active site of the antibody. It was also apparent, that ATB allowed for antibody binding with slightly greater antibody affinity, most likely due to the amine functionality used for binding. However, due to the intense, broad, multi-band nature of the SERS spectrum of ATB, it was chosen as the second best crosslinking agent, after MMT.

Using these newly developed protein sensors, the next step was to investigate their ability to detect the binding of the protein (antigen) of interest, as well as the spectral bandwidth of the resulting peaks, thereby determining the maximum number of proteins that can be simultaneously detected. From these studies, it was found that interleukin II (IL-2) and phosphorylated Zap-70 can be detected from individual nanosensors and in a label-free fashion,

at sub-nanomolar concentrations in cellular environments, following excitation with a 10mW HeNe laser. Figure 4 shows the SERS spectrum of an individual IL-2 nanosensor in cellular environments with and without nanomolar concentration equivalents of IL-2. This concentration equivalent corresponds to only a few molecules of the protein in a cell. The lower spectrum in Figure 4 represents the nanosensor prior to IL-2 introduction, while the upper spectrum (which has been offset for clarity) represents that same sensor after introduction of the cellular level concentration of IL-2. From these spectra the relative peak height of several SERS active bands between the protein-free spectrum and the IL-2 bound spectrum can be seen to change. These changes are indicative of IL-2 binding, and are highly reproducible from analysis-to-analysis and sensor-to-sensor and are due to both the addition of overlapping SERS spectral bands associated with the protein binding as well as conformational changes in the antibody upon binding.

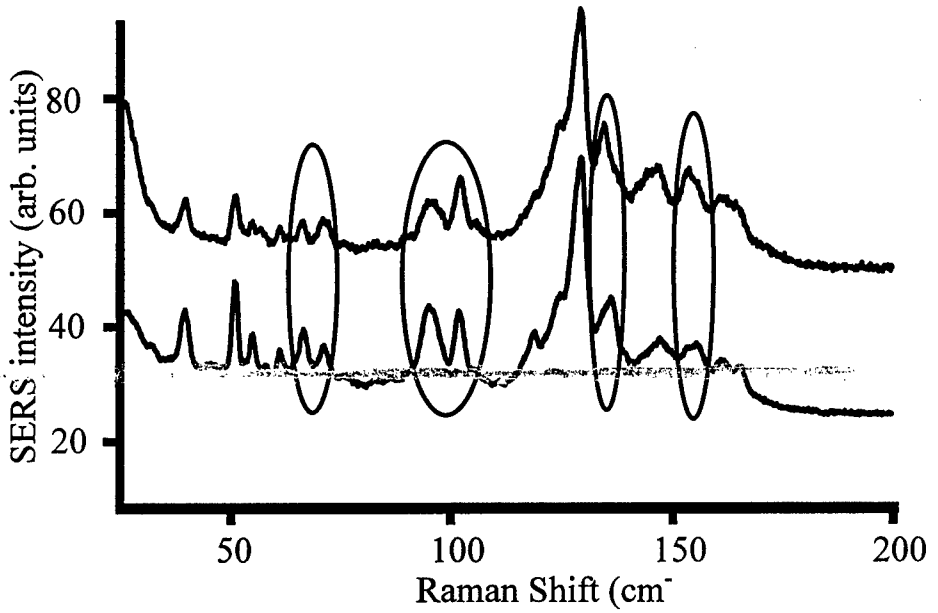

Figure 4: SERS spectra of an individual immuno-nanosensor for IL-2 in a cellular environment, a) before binding IL-2 and b) after binding IL-2.

In addition, other proteins such as insulin (a possible interferent), were also evaluated, and found to be capable of being detected with SERS immuno-nanosensros developed for these proteins and have similar detection limits, thereby providing the sensitivity necessary for intracellular monitoring of individual proteins. However, due to the specificity of monoclonal antibodies, and the highly localized SERS enhancement at the metal surface, no observable cross-reactivity was found upon exposure of each different type of sensor to complex cellular environments, during these interference studies. Figure 5 shows the SERS spectrum resulting from an individual nanosensor for insulin in a 10 nM solution of human insulin. This spectrum

was recorded using an epi-fluorescence microscope for signal collection following excitation with a 10 mW HeNe laser and detection using an intensified charge coupled device (ICCD) camera. From this spectrum, it can also be seen that the spectral linewidth of the prominent SERS band associated with insulin, at 992 cm^{-1}, is approximately 15 cm^{-1} wide (with the actual spectral line-width being even narrower than the measured value due to the resolution of the system used to obtain the measurement). This bandwidth, which is similar to the bandwidths of the relatively few bands associated with IL-2, coupled with the spectral range over which the multispectral imaging system will work (~ 300 nm) results in the ability to measure as many as 100 different protein signatures simultaneously within a cellular environment. With such a large number of potential protein signatures, the multiplexed ability of such a detection system could provide unparalleled sensitivity as well as accuracy, resulting in a low false positive rate.

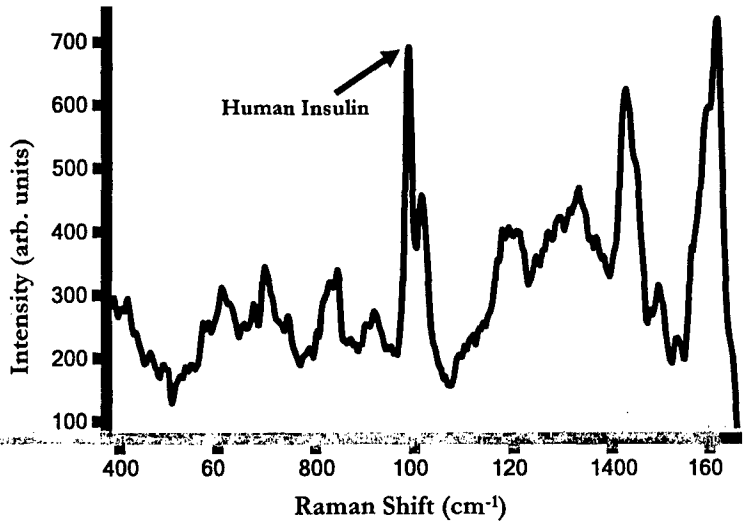

Figure 5: SERS spectrum of 10nM insulin using a SERS nanosensor

The second main thrust of this seedling effort was in the development and evaluation of a NIR optical tweezers/multispectral imaging system for the real-time positioning and monitoring of the different optical sensors developed. This work was carried out at the Army Research Laboratory (ARL) by the Co-PI (Alexander). This system consists of a 787 nm diode laser that allows for trapping of the individual nanosensors along with excitation and detection of the resulting SERS spectra. For monitoring the resulting multi-spectral images of the nanosensors in the cells, an acousto-optic tunable filter (AOTF) is used for wavelength selection. This AOTF operates over the spectral range of 500 - 900 nm, with 7 cm^{-1} spectral resolution, allowing for the rapid switching between one wavelength and another. Using this integrated optical tweezers/multispectral imaging system, it was found that the maximum silver layer thickness that

can still allow for the trapping of the individual nanosensors is approximately 300 linear nanometers. This system employs a unique optical trapping geometry, in which the center of the NIR beam used for trapping is blocked out to allow for trapping of the opaque or semi-opaque nanosensors developed in this work. Using this combined optical trapping and multispectral imaging system, background Raman/SERS signals associated with the cellular environments were evaluated and minimized via judicious selection of the excitation wavelength emplyed for these sensors, as well as the laser power that was employed. Upon optimization, only a couple of peaks were found to persist that arose from the cellular environment, providing a relatively background free spectral range over which SERS protein signatures can be measured. This is important, as it means that a larger number of proteins can be monitored simultaneously than if a large number of background bands were present.

The final area of this project involved the culturing of the CD4+ T lymphocytes and the characterization of their response to different stimuli through both conventional bioassays, and our implantable nanosensors. This work was performed in concert with the Co-PI (Ostrand-Rosenberg) and her group, who cultured the appropriate hybridoma T-cell lines employed, and confirmed results of the protein expression of these cells following exposure to SEB. In order to verify the results obtained with the newly developed implantable nanosensors, independent measurements of IL-2 expression during T-cell activation with SEB were performed using conventional bulk cellular analyses with traditional enzyme liked immunosorbent assays (ELISA) assays. These studies were run along side the nanosesor based analyses, at different doses of SEB exposure. In both cases, dose dependent expression levels of IL-2 were measured, with IL-2 production increasing in both analyses with larger doses of SEB.

Results from all aspects of this seedling project suggest the feasibility of these implantable nanosensors for the real-time monitoring of as many as 100 different cellular signaling species simultaneously. Using the current AOTF-based multispectral imaging/optical tweezers system developed in this seedling project, these analyses can be computer controlled and extremely rapid. This seedling effort, has accomplished all of the goals of this project, and far exceeded the sensitivity and rapid detection abilities expected with the discovery of a novel multi-layer SERS sensing geometry capable of enhancing SERS signals by greater than an order

of magnitude over previous single layered nanosensors developed in the PI's laboratory. Based upon these results, this technology appears to hold a great deal of promise for the early detection and differentiation of exposure to toxins and bacterial agents. By using such a system, the inherent redundancy and specificity of T-cells and the immune system should provide a detection system capable of rapid detection and differentiation of disease with a low rate of false positives, which would be of great interest to the U. S. Air Force as well as the entire United States Department of Defense. Additionally, this type of analysis system could be used as either a point detection system composed of a dish of cultured cells, or potentially, as an implantable sensing technology for the early monitoring and diagnosis of individual exposure and response biological agents, resulting in increased survivability and of exposed individuals or animals.

IV. Personnel Supported

To accomplish these goals, the research team was composed of a multi-disciplinary group of researchers (i.e., biologists, chemists and physicists), each having distinct areas of expertise necessary for the success of this project, including: nano-biosensor development, multispectral imaging, spectroscopic analyses in cellular environments, optical tweezers design and application and immunological analyses of toxin-induced cellular responses. A list of the personnel involved in this project, along with their title and contribution to the project is provided below.

- *Dr. Brian Cullum; (Faculty - UMBC)* was the PI of the project and was responsible for the direction of three graduate students in his group who worked on the development and optimization of the protein nanosensors nanosensors. In addition, he was also responsible for coordinating the various efforts of the multidisciplinary research team.
- *Dr. Troy Alexander; (Staff Scientist – ARL)*; a member of the environmental sensing team at ARL was responsible for constructing the optical tweezer/SERS detection system for positioning the SERS nanosensors.
- *Dr. Suzanne Ostrand-Rosenberg; (Faculty – UMBC)* was responsible for directing the culturing of hybridoma T-cells, as well as analysis of cellular response to external stimuli via conventional bioassay techniques.

- *Ms. Ginny Clements; (Staff, Biological Technician – UMBC)* was responsible for the day-to-day culturing and maintaining of cell lines, as well as performing stimulus tests on the T-cells.
- *Mr. Hongang Li; (Graduate student – UMBC)* was involved in the development and characterization of the SERS based protein sensors, and the development of the multilayer based SERS substrates developed in this work.
- *Ms. Mikella Hankus; (Graduate Student – UMBC)* was involved in the development of novel SERS substrate geometries for application to the SERS-based protein sensors.
- *Ms. Jian Sun; (Graduate Student – UMBC)* was involved in the development of methods for the production of the monodisperse silica nanospheres use din the fabrication of the protein nanosensors.

V. Publications

The work from this nine month seedling effort has resulted in the publication of five articles in high-quality, peer-reviewed scientific journals and two book chapters on SERS-based optical nanosensors for cellular analyses during the duration of the seedling effort, and the submission of three additional articles to high-quality, peer-reviewed journals in the coming months. These articles and book chapters are listed below.

Peer-Reviewed Journal Articles

1. M. Hankus, G. Gibson, H. Li. And B. M. Cullum, "SERS Nanoimaging Probe for Nanometer Resolution Chemical Imaging," submitted to Science (2005).
2. H. Li and B. M. Cullum, "Implantable SERS Immuno-nanosensors for the Direct Detection of Intracellular Protein Expression in Living Cells," submitted to Anal. Biochem. (2005).
3. H. Li, J. Sun, S. Ostrand-Rosenberg and B. M. Cullum, "Implantable SERS nanosensors for presymptomatic detection of BW (Biological Warfare) agents," submitted to SPIE (2005).
4. H. Li and B. M. Cullum, "Multilayer Enhancements from Continuous Silver Films Based Surface-Enhanced Raman Scattering (SERS) Substrates," Appl. Spectrosc. **58** (4), p. 13 - 20 (2005).
5. M. Hankus, G. Gibson, N. Chandrasekharan and B. M. Cullum, "Surface Enhanced Raman Scattering (SERS) – Nanoimaging Probes for Biological Analysis," SPIE, Vol. **5588**, p.106-117, (2005).
6. H. Li, P. Patel and B. M. Cullum, "Novel multilayered SERS substrates for trace chemical and biochemical analysis," SPIE, Vol. **5588**, p.87-98, (2005).
7. H. Li, J. Sun and B. M. Cullum, "Nanosphere-based SERS immuno-sensors for protein analysis," SPIE, Vol. **5588**, p.19-31, (2005).
8. H. Li and B. M. Cullum, "Development and Characterization of SERS-based Immuno-Nanosensors for Single Cell Analyses," SPIE, Vol. **5261**, p.142-154, (2004).

Book Chapters

1. B. M. Cullum, "Nanoscale Optical Biosensors and Biochips for Cellular Diagnostics," in Smart Biosensor Technology; New York, CRC Press, in press 2005.
2. B. M. Cullum, "Optical Nanosensors and Nanobiosensors," in Encyclopedia of Nanoscience and Nanotechnology; New York, Marcel Dekker, Inc., 2004, pp. 2757 - 2768.

VI. Interactions/Transitions

Presentations at Meetings

This research related to this project has resulted in the presentation of seven talks at national conferences on analytical chemistry (H. Li and B. M. Cullum, "Implantable SERS nanosensors for presymptomatic detection of BW (Biological Warfare) agents," SPIE Defense Sciences Conference, Orlando, FL., March 2005; M. Hankus, G. Gibson, N. Chandrasekharan and B. M. Cullum, "Surface Enhanced Raman Scattering (SERS) – Nanoimaging Probes for Biological Analysis," SPIE Optics East Symposium, Philadelphia, PA, October 2004; H. Li, P. Patel and B. M. Cullum, "Novel multilayered SERS substrates for trace chemical and biochemical analysis," SPIE Optics East Symposium, Philadelphia, PA, October 2004; H. Li, J. Sun and B. M. Cullum, "Nanosphere-based SERS immuno-sensors for protein analysis," SPIE Optics East Symposium, Philadelphia, PA, October 2004; H. Li and B. M. Cullum, "Development and Characterization of SERS-based Immuno-Nanosensors for Single Cell Analyses," SPIE Optics East Symposium, Providence, RI, October 2003; H. Li, N. A. Villalba Costa, and B. M. Cullum, "Novel multilayered silver film SERS substrates for trace analyses," PITTCON, Chicago, IL., March 2004; and H. Li and B. M. Cullum, "Development and optimization of nanosphere-based SERS immuno-sensors," PITTCON, Chicago, IL., March 2004). Additionally results from this work have also been included in an invited presentation by the PI (Brian Cullum) to Eli Lilly and Company in August 2004. In addition, two more presentations involving results obtained from this project have been accepted for presentation at the SPIE Optics East Symposium to be held in October.

Consulting

Over the summer of 2004 (during a portion of the time encompassed by this seedling effort), the PI (Brian Cullum) served as a project consultant for the NATO Science for Peace program. His role in this consulting effort was to act a project advisor to the project PI's (Drs. Igor Nabiev and Vladimir Oleinikov of the Universite de Reims Champagne-Ardenne in France and the

Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry in Moscow, respectively) on their project entitled "Ultrasensitive Quantum Dots-Based Biosensors." This work involved overseeing the planning of a project by the PI's on the development of sensitive quantum dot based sensors for the detection of proteins and other biological species of interest for peace. The second portion of this role as consultant, involved reporting to NATO on the ability of the project team to accomplish their goal with the resources and time available. This consulting required Dr. Cullum to travel to Moscow this past August as well as NATO headquarters in Brussels this past October to report on the project. In addition, if the project is funded, it is expected that Dr. Cullum will continue this consulting effort over the next several years, with annual trips to visit the researchers in Moscow and France as well as NATO headquarters to report on their progress.

In addition to the PI's consulting during this project's duration, the Co-PI (T. Alexander) has served as a technical advisor for a new multi-university research initiative (MURI), sponsored by the Army Research Office, on plasmonics-based instrumentation for biology. The central purpose of the MURI is to develop a novel toolset for the life scientist based on nanoplasmonics. The range of applications related to this MURI is broad, ranging from chemical/biological detection to the imaging of biological macromolecules, viruses, etc, as well as "superlens" development based on nanostructured left handed materials.

Transition of Technology

Due to the short duration of the seedling effort, no transitioning of technology or methodologies to other entities has yet occurred. However, the PI is currently speaking with individuals from two different companies (Eli Lilly and Company as well as ITT Industries) about application of technologies similar to those being developed for this project to both pharmaceutical and defense related applications. In the case of Eli Lilly and Company, the PI (Brian Cullum) has received additional funding to pursue similar implantable sensors for monitoring drug transport in individual cells. In the case of ITT Industries, the PI is currently working with them on the development of novel blue light enhanced SERS substrates, as well as continuing discussions with ITT on the development of SERS-based nano-imaging sensors for the rapid detection and identification of biological aerosols.

VII. New Discoveries

Work associate with this project has resulted in the discovery of a novel multi-layered SERS substrate geometry that can be applied to SERS substrates of almost any structural dimensionality, and is capable of enhancing the SERS signals from those geometries by over an order of magnitude. This discovery has resulted in the filing of a provisional patent entitled" Multilayered Surface Enhanced Raman Scattering (SERS) Substrates," with the U.S. Patent and Trademark Office April 26th, 2004. The provisional patent reference number is 2447BC.

VIII. Honors/Awards

During the nine month duration of this project, no new awards or honors were bestowed upon the personnel supported.

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The goal of this project was to develop and demonstrate the potential of a versatile class of nano-biosensors capable of being implanted within individual living cells for the direct conversion of biochemical signaling events into electronic information. In order to monitor the various biochemical species responsible for intracellular signaling (e.g., proteins), with minimal stress to the cell during analysis, we are developing nanobead sensors between 200 and 500 nm in diameter that are capable of being non-invasively positioned at different locations within a living cell via optical tweezers. These sensors employ surface enhanced Raman spectroscopy (SERS) and allow for the qualitative as well as quantitative monitoring of the expression of the specific proteins required for cellular response. Monitoring intracellular signaling species in this fashion allows for the detection of the earliest possible signals associated with a stimulation event, as well as allow for the differentiation of various events from one another at the earliest possible time.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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